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A Mutation in a 3-Keto-Acyl-ACP Synthase II Gene is Associated with Elevated Palmitic Acid Levels in Soybean Seeds

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ABSTRACT

Palmitic acid is the major saturated fatty acid component of soybean [*Glycine max* (L.) Merr.] oil, typically accounting for approximately 11% of total seed oil content. Several genetic loci have been shown to control the seed palmitate content of soybean. One such locus, *fap$_2$*, mediates an elevated seed palmitate phenotype. Previous biochemical studies indicated that the *fap$_2$* locus is associated with a reduction in the activity of 3-keto-acyl-ACP synthase II (KAS II), an enzyme that initiates the elongation of palmitoyl-ACP to stearoyl-ACP in the plastid. The objective of the present research was to define the molecular basis by which the *fap$_2$* locus increases seed palmitate levels. We isolated two closely related, yet unique *KAS II* cDNAs, designated *GmKAS IIA* and *GmKAS IIB,* from soybean cultivar Century (*Fap$_2$, Fap$_2$*) and its derivative high palmitate germplasm C1727 (*fap$_2$, fap$_2$*). The *GmKAS IIB* cDNAs recovered from Century and C1727 were identical. In contrast, a single base-pair substitution was found in the *GmKAS IIA* gene from C1727 versus Century which converted a tryptophan codon into a premature stop codon, a mutation that would be predicted to render the encoded enzyme nonfunctional. Knowledge of the DNA sequence polymorphism led to the development a facile, robust cleavage amplified polymorphic sequence (CAPS) marker that readily distinguishes the mutant *GmKAS IIA* gene. This marker faithfully associated with a second independent germplasm line bearing the *fap$_2$* locus, and thus may be useful in breeding programs that target the development of high palmitate soybean cultivars.

Soybean oil stability and quality is primarily determined by its fatty acid composition. Of particular importance is the relative proportion of saturated versus unsaturated fatty acids (Liu and White, 1992; Shen et al., 1997). A comprehensive understanding of the genetic and metabolic factors that control seed oil composition would greatly enhance our ability to develop soybean germplasm with oil compositions optimized for specific applications. Such applications include (i) increasing the content of nutritionally favorable fatty acids while decreasing those that are considered detrimental; (ii) increasing the total oil content; and (iii) enhancing oil stability by increasing the content of saturated fatty acids.

Palmitic acid (palmitate, 16:0) is the major saturated fatty acid component of soybean seed oil, typically averaging 11% (w/w) of total seed oil in standard soybean cultivars. Efforts to breed soybean with altered palmitate composition have yielded germplasm lines with both low (4%) and elevated (40%) palmitate (Stoltzfus et al., 2000a; Pantalone et al., 2004). The effort to establish low palmitic acid cultivars is largely driven by the U.S. Food and Drug Administration goal of producing edible oil with <7% total saturates, due the cholestrogenic nature of saturated fats. However, soybean oils with low or normal 16:0 content and high in polyunsaturated fatty acids are unstable at higher temperatures, and for many applications must be hydrogenated during processing. This process leads to the generation of *trans*-fatty acids, which have been established as a health risk (Hu et al., 1997; Katan, 1998). The development of high palmitate germplasm may provide a means to improve the oxidative stability of soybean oil, as well as help enable the production of *trans*-free solid or semisolid fats used in the manufacture of shortenings and margarine (Kok et al., 1999).

Genetic loci that harbor mutations leading to altered palmitic acid phenotypes are designated *Fap*. Low palmitate phenotypes are exhibited by lines carrying chemically induced recessive mutations at loci designated *fap$_1$*, *fap$_3$*, or *fap** (Erickson et al., 1988; Schnebly et al., 1994; Wilcox et al., 1994; Stojsin et al., 1998). In addition, a naturally occurring low palmitate germplasm carries a mutation in a locus designated *fap$_{nc}$* (Burton et al., 1994). *fap$_3$*, *fap**, and *fap$_{nc}$* are not allelic to *fap$_1$*, but it is not known if they are allelic to one another (Fehr et al., 1991). High palmitate phenotypes are exhibited by lines carrying chemically induced recessive mutations at loci designated *fap$_2$*, *fap$_{2b}$*, *fap$_4$*, *fap$_5$*, *fap$_6$*, *fap$_7$*, and *fap$_x$* (Wilcox and Cavins, 1990; Schnebly et al., 1994; Narvel et al., 2000; Stoltzfus et al., 2000b, 2000c; Rahman et al., 1999, 2003). Inheritance data reported to date have only revealed close linkages (and thus potential allelism) among the *fap$_2$, fap$_{2b}$*, and *fap$_5$* loci, and between *fap$_6$* and *fap$_7$* (Narvel et al., 2000; Stoltzfus et al., 2000b, 2000c).

The molecular mechanism by which each *fap* locus controls palmitate levels is largely unknown. Genetic loci that harbor genes encoding fatty acid biosynthetic and metabolic enzymes are likely to be directly involved in controlling palmitate levels. As shown in Fig. 1, de novo fatty acid biosynthesis is initiated within the plastid by the carboxylation of acetyl-CoA to malonyl-CoA, catalyzed by the enzyme acetyl-CoA carboxylase. Following this conversion, individual enzymes of the fatty acid synthase complex catalyze a series of condensation, reduction, and dehydration reactions leading to the formation of palmitoyl (16:0)-ACP (reviewed in Ohlrogge

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Abbreviations: ACP, acyl carrier protein; CAPS, cleaved amplified polymorphic sequence; EMS, ethyl methane sulfonate; ESTs, expressed sequence tags; FAT, fatty acid thioesterase; KAS, 3-keto-acyl-ACP synthase.

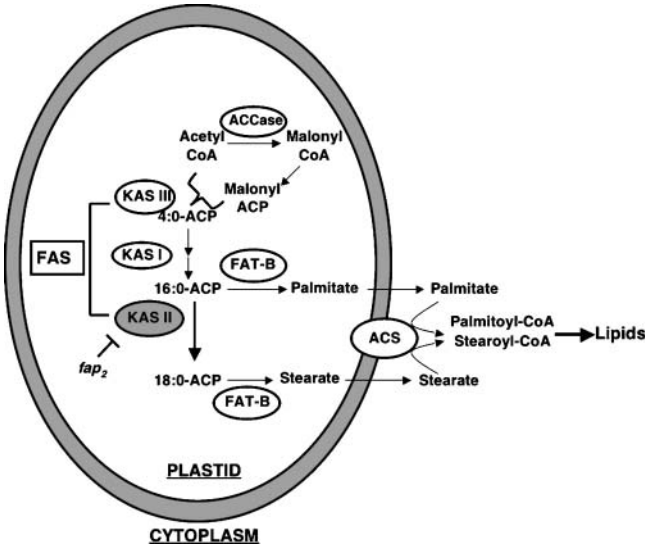

Fig. 1. Schematic representation of the steps of the fatty acid pathway in plants involved with saturated fatty acid biosynthesis. Key enzymes in the pathway are depicted in elliptical boxes. The condensation enzyme KAS II is part of the fatty acid synthase (FAS) complex. The mutant *fap*$_2$ locus is believed to be the result a debilitating mutation in a *KAS II* gene. ACC, acetyl Co-A carboxylase; FAT-B, fatty acid thioesterase B; ACS, acyl-CoA synthase.

and Browse, 1995). A special condensing enzyme, 3-ketoacyl-ACP synthase II (KAS II) is required to initiate the elongation of 16:0-ACP to 18:0-ACP, a reaction that competes with the 16:0-ACP fatty acid thioesterase (FAT-B) enzyme that cleaves the fatty acid from its carrier to yield free palmitic acid (Fig. 1).

In a previous study, in vivo acetate saturation kinetic analyses suggested that soybeans homozygous for the *fap*$_2$ locus displayed reduced KAS II activity (Wilson et al., 2001a, 2001b). A direct association between KAS II activity and palmitate levels has also been demonstrated in the model plant species *Arabidopsis.* The *fab* mutation of *Arabidopsis* is defined by a mutation in a *KAS II* gene that severely impairs the condensation activity of the enzyme, resulting in plants displaying an elevated palmitate phenotype (Carlsson et al., 2002). In this study we characterize two soybean *KAS II* genes, designated *GmKAS IIA* and *GmKAS IIB*, in a normal (*Fap*$_2$*Fap*$_2$) and high palmitate (*fap*$_2$*fap*$_2$) genetic background. A debilitating point mutation was discovered in the *GmKAS IIA* gene of plants possessing the *fap*$_2$ locus.

MATERIALS AND METHODS

Plant Materials

Soybean genotypes Century, C1727 and N02–4441 were used in this study. Century (*Fap*$_2$, *Fap*$_2$) exhibits a normal palmitate phenotype (approx. 11% 16:0). The high palmitic acid (16.2% 16:0) germplasm C1727 (*fap*$_2$, *fap*$_2$) originated as an M_2 selection from a population of Century seeds treated with the mutagen ethyl methane sulfonate (EMS) (Wilcox and Cavins, 1990). N02–4441 (*fap*$_2$, *fap*$_2$) is a high palmitate (15.6% 16:0) F_3–derived F_5 line selected from a cross between PI424182-B and Cx1537–53. Cx1537–53 (*fap*$_2$, *fap*$_2$) is a high palmitate selection derived from a cross between CX1334–352 and C1727 (*fap*$_2$, *fap*$_2$) (Dr. Jim Wilcox, personal communication, 25 Feb. 2005).

Nucleic Acid Isolation and Analysis

Genomic DNAs were isolated from 2- to 4-g frozen leaf tissue as previously described (Dewey et al., 1994). For Southern blotting assays, 10 μg of genomic DNA was digested with restriction endonucleases *Bam*HI, *Hin*dIII, or *Xba*I (Promega Corporation, Madison, WI) at 37°C for 4 h. Digested DNA was separated by electrophoresis in a 1% agarose gel in Tris-phosphate-EDTA buffer and transferred to nylon membrane (Sambrook and Russell, 2001). Blotted DNA was hybridized against a ^{32}P-labeled probe corresponding to a 1000-bp *Eco*RI fragment of the soybean *KAS II* cDNA sequence represented in GenBank (AF244518) and washed according to standard protocols (Sambrook and Russell, 2001). Radioactive blots were analyzed by exposure to BioMax X-ray film (Eastman Kodak, Rochester, NY).

Total celluar RNA was isolated from frozen leaf tissues by the Trizol method according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). First strand cDNA was generated from 20 μg total RNA via reverse transcription (Superscript II, Invitrogen) using either an oligo-dT_{18} primer or the dT-AP primer (Invitrogen).

Isolation of *GmKASII* cDNA and Genomic Sequences

To amplify full-length *KAS II* cDNAs from Century and C1727, primers were originally designed based on a soybean *KAS II* sequence deposited in Genbank (accession number AF244518). Specifically, primers 5′-CAACCACACCCTTGGATTTA-3′ and 5′-ATGGTAGTGGATAGATAGCG-3′ were successful in amplifying a full-length version of *GmKAS IIA* from Century and C1727. Amplification reactions were performed using the Expand Hi-fidelity PCR system (Roche Applied Science, Indianapolis, IN). Each 50-μL reaction contained 10 m*M* Tris-Cl (pH 8.3), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 200 μ*M* each dNTP, 25 pmol of each primer, and 1 to 2 μL of the appropriate first-strand cDNA as template. Thirty amplification cycles, each composed of denaturing at 94°C for 30 s, annealing at 45°C for 30 s, and extension at 72°C for 2.5 min, were followed by a final 7-min extension step at 72°C.

Full-length sequence information for *GmKAS IIB* was obtained by combining the results of 3′ and 5′ rapid amplification of cDNA ends (RACE) reactions. For 3′ RACE-PCR, first strand cDNA was synthesized using primer dT-AP (Invitrogen), and PCR was performed using the primer 5′-ATGGGGCTGGTGTTATTCTT-3′ together with the dT-AP primer. For 5′ RACE-PCR, primers 5′-CAACCACACCCTTGGATTTA-3′ and 5′-AATGCCTTTTCAATGCAAAGA-3′ were used. The amplification conditions for both 5′ RACE and 3′ RACE-PCR were identical: 35 cycles of 30 s denaturation at 94°C, 30 s annealing at 51°C, and 2 min extension at 72°C.

Amplification of an approximately 1000-bp genomic sequence surrounding the mutated region of *GmKAS IIA*, in addition to the corresponding region of *GmKAS IIB*, was conducted using primers 5′-AGGCTCAGATGCTGCTAT-3′ and 5′-CATGCTCCAGTTCCTCTAA-3′. The PCR conditions were the same as described for amplification of the full-length *GmKAS IIA* cDNA with the exception of using 12.5 ng of genomic DNA as template and an extension temperature of 50°C. All genomic and cDNA amplification products described above were cloned into the pCR 2.1 cloning vector (Invitrogen) and sequenced using the DNA Facility at Iowa State University (www.dna.iastate.edu).

CAPS Marker Analysis

CAPS assays were conducted using the *GmKAS IIA*-specific primers 5′-GGCTTAAGCGTAGTTATCATG-3′ and 5′-TTCAAAGTGTCCATGCCTAAC-3′. PCR conditions were the same as described above for the genomic DNA amplification with the exception of using an extension temperature of 52°C. Following amplification, PCR products were purified using QIAquick columns (Qiagen Corp., Valencia, CA) and digested with the restriction enzyme *Dde*I (Promega). Ten microliters of each PCR reaction mix was incubated for 1.5 h at 37°C in a 20-μL reaction containing 10 units of *Dde*I along with buffer components supplied by the manufacturer (Promega). DNA fragments were subsequently separated on a 4% agarose gel (Agarose SFR, Amresco, Solon, OH) in Tris-Borate-EDTA buffer (Sambrook and Russell, 2001). The ethidium bromide–stained DNA was then visualized using an Alpha Imager (Alpha Innotech, San Leandro, CA).

RESULTS

Characterization of *KAS II* Genes in Century and C1727

The results of a previous in vivo acetate saturation kinetic analysis suggested that developing seeds of line C1727 possessed decreased KAS II activity (Wilson et al., 2001b). Because germplasm C1727 was derived via EMS mutagenesis of cultivar Century (Erickson et al., 1988), the most straightforward means by which KAS II activity could be reduced would be through a disabling mutation in a *KAS II* gene. To assess whether gross differences in *KAS II* gene structure were apparent between genomic DNAs of Century versus C1727, a Southern blot analysis was conducted using a radiolabeled soybean *KAS II* cDNA as a hybridization probe (GenBank Accession number AF244518). As shown in Fig. 2, multiple hybridizing bands were observed, regardless of the restriction enzyme used. This result suggests that the soybean genome encodes more than one distinct *KAS II* gene. The failure to observe differences in the banding patterns between Century and C1727 suggests that no major structural differences, such as a deletion, insertion, or rearrangement, exist between the *KAS II* genes of the two soybean lines.

EMS treatment typically results in G-residue alkylation, leading to G→A or C→T transition mutations (Anderson, 1995). To test whether point mutations occur in a *KAS II* gene from C1727, in comparison to the same gene from Century, sequence information from the soybean *KAS II* gene found in GenBank at the time this study was initiated (AF244518) was used to design *KAS II*-specific PCR primers. Utilization of these primers, and additional primers described in Materials and Methods, led to the recovery of two highly homologous, yet clearly distinct, *KAS II* cDNAs from Century that we have designated *GmKAS IIA* and *GmKAS IIB*. These sequences have been deposited in GenBank as accession numbers AY907523 (*GmKAS IIA*) and AY907522 (*GmKAS IIB*). *GmKAS IIA* and *GmKAS IIB* share 97.6% nucleotide sequence identity and 97.5% identity at the predicted protein level. Although *GmKAS IIA* and *GmKAS IIB* were recovered using PCR primers designed against the soybean sequence AF244518, several polymorphisms were observed among *GmKAS IIA*, *GmKAS IIB*, and AF244518, suggesting that each may represent unique isoforms within the soybean genome. Conceptual translations of *GmKAS IIA*, *GmKAS IIB*, and AF244518 are shown in Fig. 3. The predicted amino acid sequence of AF244518 is 98 and 95.9% identical to the GmKAS IIA and GmKAS IIB proteins, respectively.

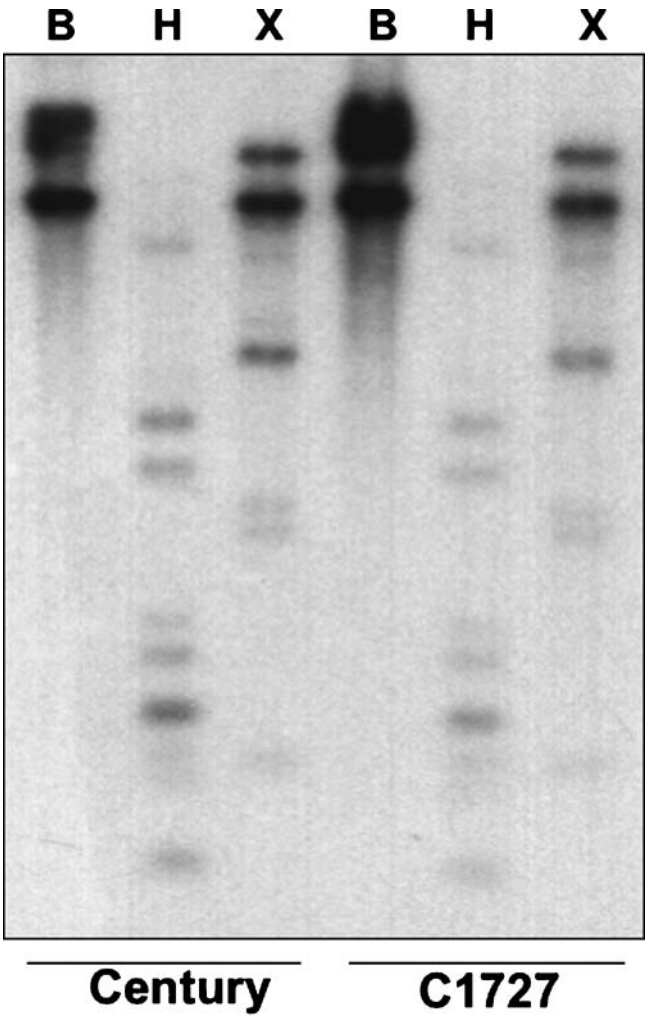

Fig. 2. DNA gel blot analysis of *GmKAS II* genes in soybean. Genomic DNAs from Century (*Fap*$_2$, *Fap*$_2$) and C1727 (*fap*$_2$, *fap*$_2$) were digested with the restriction enzymes *Bam*HI (B), *Hin*dIII (H), and *Xba*I (X). Blotted DNAs samples were hybridized to a ^{32}P-labeled probe corresponding to a fragment of a soybean *KAS II* gene (AF244518).

As a means of further assessing the complement of *KAS II* genes that are expressed in the soybean genome, an in silico analysis was conducted on the soybean expressed sequence tags (ESTs) that have been deposited in GenBank. The public soybean genome initiative has been responsible for generating more than 286 000 ESTs derived from 80 soybean cDNA libraries representing a variety of developmental stages, tissue types, and treatments (Shoemaker et al., 2002). BLASTN analyses revealed 26 soybean ESTs with high homology to *KAS II* (Table 1). Inspection of each individual alignment enabled us to categorize 14 ESTs as corresponding to *GmKAS IIA* and 10 ESTs as corresponding to *GmKAS*

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 AF244518    (1) MASTTTSSLCTWLVAACMSVTCHADRTKTPHAMFRSSKKSRYSQFNVCRS
GmKAS IIA    (1) ......................H....K....M........Y.....C..
GmKAS IIB    (1) ......................D....R....I........K.....S..

 AF244518   (51) THSGKTMAVALQPTQGITTIKKPPTKQRRVVVTGLGVVTPLGHEPDIFYN
GmKAS IIA   (51) ................EI................................
GmKAS IIB   (51) ................EV................................

 AF244518  (101) NLLDGASGISEIETFDCADIPTRIAGEIKSFSTDGWVAPKLSKRMDKFML
GmKAS IIA  (101) .....A............EY..............................
GmKAS IIB  (101) .....V............EY..............................

 AF244518  (151) YMLTAGKKALVDGGITDDVMDELYKDKRGVLIGSAMGGMKVFNDAIEALR
GmKAS IIA  (151) ......................N.E.C.....................
GmKAS IIB  (151) ......................N.D.C.....................

 AF244518  (201) ISYKKMNPFCVPFATTHMGSAMLAMDLGWMGPNYSISTACATSNFCILNA
GmKAS IIA  (201) ................N...............................
GmKAS IIB  (201) ................N...............................
                                                               *
 AF244518  (251) ANHIIRGEADVMLCGGSDAAIIPIGLGGFVACRALSQRNTDPTKASRPWD
GmKAS IIA  (251) ................................................
GmKAS IIB  (251) ................................................

 AF244518  (301) INRDGFVMGEGAGVLLLEELEHAKERGATILAEFLGGSFTCDAYDVTEPR
GmKAS IIA  (301) ......................E.....Y.............H.....
GmKAS IIB  (301) ......................K.....Y.............H.....

 AF244518  (351) PDGAGVILCIEKALAQSGVSKEDVNYINAHATSTPAGDLKEYQALMHCFG
GmKAS IIA  (351) ................................................
GmKAS IIB  (351) ................................................

 AF244518  (401) QNPELRVNLTKSMIGHLLGAAGGVEAVATIQAIRTGWVHPNINLENPDNG
GmKAS IIA  (401) ...E....S.............G....................N....
GmKAS IIB  (401) ...K....S.............A....................K....

 AF244518  (451) VDAKVLVGSKKERLDVKAALSNSFGFGGHNSSIIFAPY
GmKAS IIA  (451) ...................................
GmKAS IIB  (451) ...................................K
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Fig. 3. Multiple sequence alignment of predicted soybean KAS II enzymes. AF244518 represents a full-length soybean KAS II sequence obtained from GenBank. GmKAS IIA and IIB represent the conceptual translations of the respective *GmKASII* gene sequences from cultivar Century. Dots depict amino acid residues identical among all three sequences. Shaded residues highlight dissimilar amino acids. An asterisk is located above the tryptophan residue (W) in the normal soybean sequences where the predicted protein derived from the mutant *GmKAS IIA* gene product from C1727 terminates due to the introduction of a premature stop codon.

IIB (data not shown). The remaining two ESTs could not be clearly grouped with any characterized full-length soybean *KAS II* gene and therefore may represent an additional unique isoform. Interestingly, no soybean EST was found that showed greater homology to the AF244518 sequence than to either *GmKAS IIA* or *GmKAS IIB.* Although the question of precisely how many unique *KAS II* genes are encoded by the soybean genome remains unclear, the in silico analysis suggests that transcripts corresponding to *GmKAS IIA* and *GmKAS IIB* are found in the greatest abundance.

The DNA sequence of the *GmKAS IIB* cDNA from line C1727 was identical to the sequence isolated from Century. Pairwise alignment of the cloned *GmKAS IIA* sequences from Century and C1727, however, revealed a single G→A substitution in the coding region of the C1727 sequence. This polymorphism occurs in codon 299 and results in the introduction of a premature stop codon (TAG) in the gene from C1727 where a tryptophan codon (TGG) normally resides (Fig. 3). The truncated protein that would be produced by the mutant *GmKAS IIA* gene in C1727 would lack more than a third of the residues found in wild-type KAS II enzymes and would thus be predicted to be nonfunctional. The discovery of a mutant, presumably nonfunctional *KAS II* gene in C1727 is consistent with the in vivo saturation

Table 1. GenBank accession numbers of soybean expressed sequence tags (ESTs) sharing high sequence identity with *GmKAS II* cDNAs.

GmKAS IIA-like ESTs†	*GmKAS IIB*-like ESTs†	Unique ESTs‡
BG649823	BM893353	BM731357
AW100162	BI787448	AW760422
BE020788	BI786171	
BM187858	BU551174	
BI970924	BG726756	
BI945666	BQ299042	
BU577940	BM091721	
AI496152	AW781401	
BF070720	BU764362	
CD393099	BQ785824	
BF066315		
BG239447		
BU545669		
BE023646		
14 total	10 total	2 total

† ESTs sharing >98% nucleotide sequence identity to the corresponding *GmKAS II* gene (excluding Ns and gaps).
‡ ESTs sharing <93% nucleotide sequence identity with either *GmKAS IIA* or *GmKAS IIB*.

kinetic data of our previous study that suggested that developing seeds of this line were deficient in KAS II enzyme activity (Wilson et al., 2001b).

Development of Molecular Marker Associated with the *GmKAS IIB* Mutation

The G→A mutation in codon 299 (nucleotide 896 with respect to the start ATG codon) in the *GmKAS IIA* gene from C1727 not only resulted in the introduction of a premature stop codon, it also created a new recognition site for the restriction endonuclease *Dde*I. The recognition motif of the *Dde*I enzyme is 5′-CTNAG-3′, a sequence found in the mutated region of the *GmKAS IIA* gene of C1727 (CTT*A*G) that is not present at this location in the wild-type gene from Century (CTT*G*G). The presence of a unique restriction site can be exploited to create a CAPS molecular marker. CAPS markers are developed by designing highly specific PCR primers to amplify the genomic sequence surrounding the polymorphic restriction site, followed by enzyme digestion and gel electrophoresis to reveal the polymorphism (Konieczny and Ausubel, 1993).

Our initial attempts to create an effective CAPS marker specific for the mutant *GmKAS IIA* gene were hindered by two issues: (i) the high degree of sequence similarity between *GmKAS IIA* and *GmKAS IIB* made it difficult to design primers that would exclusively yield amplification products of the former; and (ii) all amplification reactions using primers based on the *GmKAS IIA* cDNA sequence gave larger than expected products using genomic DNAs as template, suggesting the presence of introns in this region of the gene. To overcome these obstacles, oligonucleotide primers (see Materials and Methods) were designed to amplify a region spanning 320 bp of the cDNA sequences of *GmKAS IIA* and *GmKAS IIB*. These primers yielded genomic amplification products of approximately 1000 bp that were subsequently sequenced. A portion of the genomic sequences of *GmKAS IIA* and *GmKAS IIB* surrounding the region of *GmKAS IIA* that is mutated in C1727 is shown in Fig. 4A.

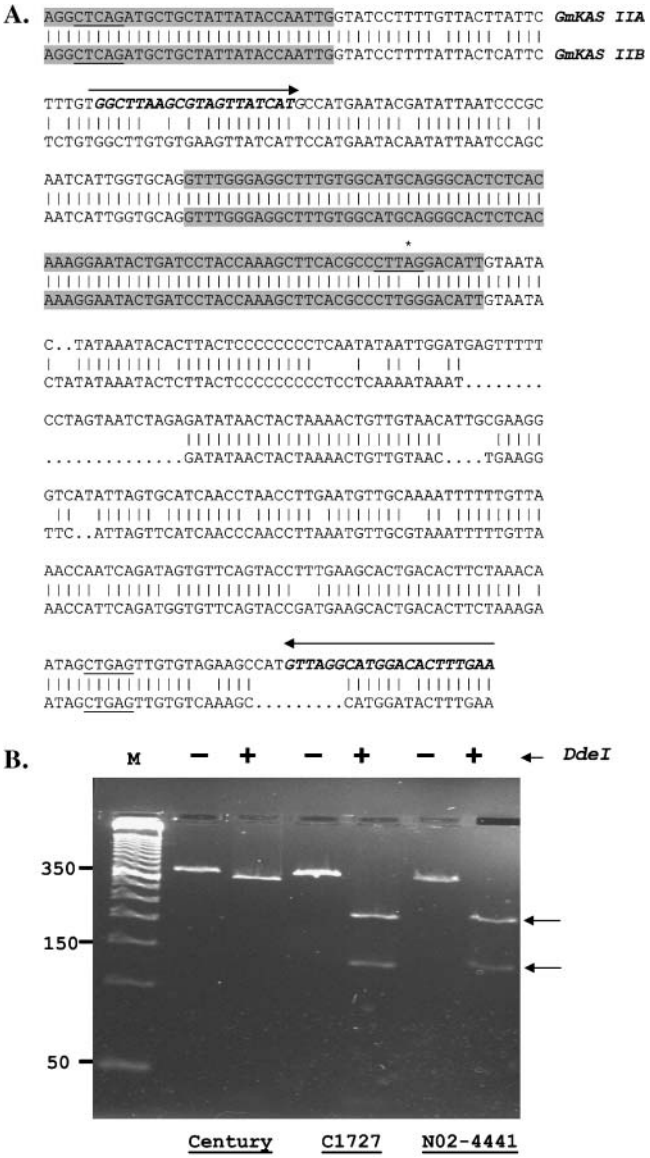

Fig. 4. CAPS analysis of normal versus mutant *GmKAS IIA* genes from soybean. (A) Partial genomic sequence of *GmKAS IIA* and *GmKAS IIB* genes from C1727 showing exon (shaded) and intron (nonshaded) sequences in the vicinity of the *Dde*I site used for CAPS analysis. The locations of primer sequences used to specifically amplify the *GmKAS IIA* isoform are shown with arrows. An asterisk is located above the polymorphic A residue that creates a unique *Dde*I site in the *GmKAS IIA* gene from C1727 that is a G residue in the corresponding gene from Century. All *Dde*I recognition sites (CTNAG) within the displayed sequences are underlined. (B) *Dde*I digestion of genomic DNAs amplified from Century (*Fap*$_2$, *Fap*$_2$), C1727 (*fap*$_2$, *fap*$_2$), and N02–4441 (*fap*$_2$, *fap*$_2$) using the primer sequences shown in (A). Arrows point to the 220- and 131-bp fragments diagnostic of germplasm possessing the mutant *GmKAS IIA* gene. M, 50-bp molecular weight ladder.

In contrast to the paucity of sequence polymorphisms observed between the coding regions of *GmKAS IIA* and *GmKAS IIB*, substantial polymorphism was apparent within intron sequences, facilitating the design of gene-specific primers (Fig. 4A). Amplification of soybean genomic DNA with the *GmKAS IIA*-specific primer pair yields a 390-bp product (Fig. 4B). *Dde*I diges-

tion of the 390-bp amplification product derived from Century generates fragments of 39 and 351 bp (in the gel system used, the 39-bp fragment is typically not seen or co-migrates with unincorporated primers). The presence of the novel *Dde*I site in the *GmKAS IIA* gene in C1727, however, enables the cleavage of the 351-bp band to fragments of 220 and 131 bp, creating an easily distinguishable molecular marker.

As further validation of the mutant *GmKAS IIA*-specific CAPS marker, we isolated genomic DNA from N02–4441 (*fap$_2$, fap$_2$*), a high palmitate selection (15.6% 16:0) derived from crosses originating with C1727 (see Materials and Methods). As shown in Fig. 4B, CAPS analysis of the N02–4441 line produced the same pattern as C1727, indicating that the mutant *GmKAS IIA* gene faithfully associates with the *fap2* genotype.

DISCUSSION

We have identified a debilitating point mutation in a *KAS II* gene isolated from a high palmitic acid soybean line that originated via chemical mutagenesis. The G→A polymorphism that results in a tryptophan codon (TGG) in the *GmKAS IIA* gene from Century being replaced by a premature stop codon (TAG) in the gene recovered from C1727 is consistent with the mode of action of EMS, a mutagen that yields transition mutations through the alkylation of G residues (Anderson, 1995). The notion that a deficiency in KAS II enzymatic activity would lead to enhanced palmitate content in the seed is consistent with our understanding of the biochemistry of fatty acid biosynthesis in plants. KAS II mediates the elongation of 16:0-ACP to 18:0-ACP. A reduction in the flow of metabolites through this step would be predicted to lead to a greater accumulation of the 16:0-ACP pool, favoring the enhanced production of palmitate via the 16:0-ACP fatty acid thioesterase enzyme (FAT-B) that competes with KAS II for the same 16:0-ACP substrate (Fig. 1). Furthermore, the discovery of a mutation in a *KAS II* gene is consistent with our previous kinetic analysis of developing seeds of line C1727, a study that predicted a deficiency in KAS II enzyme activity in this germplasm (Wilson et al., 2001b). Cumulatively, our results present compelling evidence that the genetic lesion that defines the *fap2* locus which mediates the high palmitate content in C1727 is the mutation in the *GmKAS IIA* gene described in this report. This conclusion is further supported by the appearance of the mutant *GmKAS IIA* allele in N02–4441, a soybean line whose high palmitic acid phenotype originated from C1727.

The results from this study suggest that the soybean genome encodes at least two functional *KAS II* genes, *GmKAS IIA* and *GmKAS IIB*. The fact that two soybean ESTs (accession numbers BM731357 and AW760422) and an additional full-length soybean sequence (accession number AF244518) are found within GenBank that do not clearly correspond to either *GmKAS IIA* or *GmKAS IIB* gives rise to the possibility of even more unique isoforms. In addition to *fap$_2$*, loci designated *fap$_{2b}$*, *fap$_4$*, *fap$_5$*, *fap$_6$*, *fap$_7$*, and *fap$_x$* have also been characterized that confer an elevated palmitate phenotype in the soybean seed. Although *fap$_2$* is either allelic or closely linked to *fap$_{2b}$* and *fap$_5$* (Schnebly et al., 1994; Stoltzfus et al., 2000b), *fap$_4$*, *fap$_6$*, *fap$_7$*, and *fap$_x$* segregate independently of *fap$_2$* (Schnebly et al., 1994; Stoltzfus et al., 2000c; Rahman et al., 2003). Thus, the *GmKAS IIB* gene (and/or other unique *KAS II* isoforms) would represent a good candidate target for the genetic lesion underlying one or more of these other independent *fap* loci.

In conclusion, we identified two distinct isoforms of a soybean gene encoding the enzyme KAS II, a mutation in one of which is associated with the high palmitate phenotype mediated by the *fap$_2$* locus. In addition to providing a rational biochemical explanation of the *fap$_2$* phenotype, elucidation of the point mutation in the *GmKAS IIA* gene of C1727 enabled the development of a facile, CAPS marker specific for the mutant *KAS II* allele. Utilization of this marker could assist breeding efforts designed to develop soybean cultivars with a higher saturated fatty acid content for applications where oils possessing higher oxidative stability and increased melting temperatures are desired.

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